

Amendment and Response

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Serial No.: 10/028,224

Confirmation No.: 4497

Filed: 21 December 2001

**For: CRYSTALLIZATION AND STRUCTURE DETERMINATION OF GLYCOSYLATED HUMAN BETA
SECRETASE, AN ENZYME IMPLICATED IN ANZHEIMER'S DISEASE**

Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

1-53. (Canceled)

54. (Original) A method for crystallizing a human beta secretase molecule or molecular complex comprising:

preparing purified human beta secretase in the presence of an inhibitor; and
crystallizing human beta secretase from a solution having a pH of about 3.5 to about 5.5.

55. (Previously Presented) The method of claim 59 wherein the salt is selected from the group consisting of sodium chloride, ammonium sulfate, magnesium sulfate, lithium sulfate, and combinations thereof.

56. (Original) The method of claim 54 wherein the solution has a pH of about 4.0 to about 4.7.

57. (Original) The method of claim 54 wherein the solution comprises a buffer having a pK_a of about 3 to about 6.

58. (Previously Presented) The method of claim 66 wherein the glycol is selected from the group consisting of PEG, PEG-MME, PEG-DME, polyoxyalkylenepolyamines, and combinations thereof.

59. (Original) The method of claim 54 wherein the solution further comprises a salt.

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60. (Original) The method of claim 59 wherein the salt is present in a concentration of about 0.001 M to about 0.5 M.

61. (Original) The method of claim 54 wherein the solution includes up to about 40% by weight organic solvent.

62. (Original) The method of claim 61 wherein the organic solvent is DMSO.

63. (Original) The method of claim 54 wherein the solution further comprises up to about 40% by weight ethylene glycol or glycerol.

64. (Original) The method of claim 54 wherein the beta secretase is present at a concentration of about 1 mg/ml to about 80 mg/ml.

65. (Original) The method of claim 54 wherein the inhibitor is present at a concentration of about 0.1 to about 10 mM.

66. (Original) The method of claim 54 wherein the solution further comprises about 5% by weight to about 50% by weight of a glycol.

67. (Original) The method of claim 66 wherein the glycol is a monomeric or polymeric glycol.

68. (Original) The method of claim 54 wherein the human beta secretase is isolated from mammalian cells.

69. (Original) The method of claim 68 wherein the mammalian cells are CHO-K1 cells.

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70. (Original) The method of claim 68 wherein the mammalian cells are HEK 293 cells.
71. (Previously Presented) The method of claim 54 wherein the human beta secretase is isolated from insect cells as part of a Baculovirus expression system.
72. (Original) A crystal of beta secretase having the trigonal space group symmetry $P3_221$.
73. (Original) A crystal of beta secretase comprising a unit cell having dimensions of a, b, and c, wherein a is about 77 Å to about 147 Å, b is about 77 Å to about 147 Å, and c is about 77 Å to about 147 Å; and $\alpha=\beta=90^\circ$, and $\gamma=120^\circ$.
74. (Original) A crystal of beta secretase having the trigonal space group symmetry $P3_221$ and comprising a unit cell having dimensions of a, b, and c, wherein a is about 77 Å to about 147 Å, b is about 77 Å to about 147 Å, and c is about 77 Å to about 147 Å; and $\alpha=\beta=90^\circ$, and $\gamma=120^\circ$.
75. (Original) The crystal of claim 74 having amino acid sequence SEQ ID NO:1.
76. (Previously Presented) The crystal of claim 74 having amino acid sequence SEQ ID NO:1, with the proviso that at least one methionine is replaced with selenomethionine.
- 77-78. (Canceled)
79. (New) The crystal of claim 75 wherein the crystal further comprises an inhibitor.

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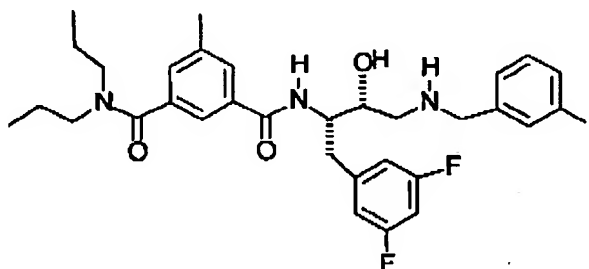
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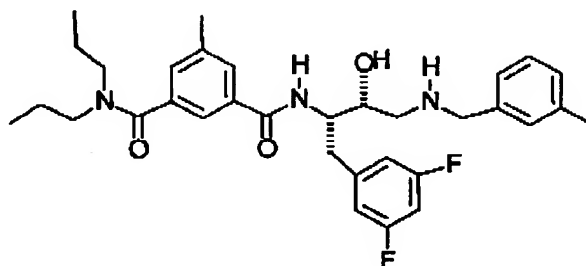
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80. (New) The crystal of claim 79 wherein the inhibitor is



81. (New) The crystal of claim 76 wherein the crystal further comprises an inhibitor.

82. (New) The crystal of claim 81 wherein the inhibitor is



83. (New) A crystal of beta secretase having amino acid sequence SEQ ID NO:1, wherein the crystal has the trigonal space group symmetry $P3_221$ and comprises a unit cell having dimensions of a, b, and c, wherein $a = b = 112 \pm 20 \text{ \AA}$, $c = 110 \pm 20 \text{ \AA}$, $\alpha = \beta = 90^\circ$, and $\gamma = 120^\circ$.

84. (New) The crystal of claim 83 wherein the crystal further comprises an inhibitor.

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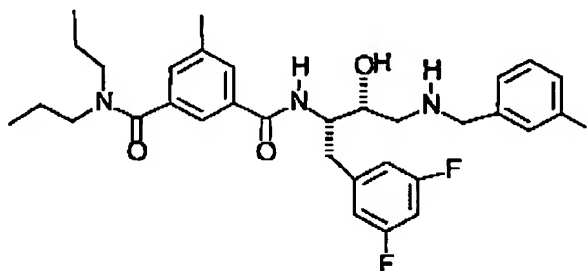
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85. (New) The crystal of claim 84 wherein the inhibitor is



86. (New) A crystal of beta secretase having amino acid sequence SEQ ID NO:1, wherein the crystal has the trigonal space group symmetry $P3_221$ and comprises a unit cell having dimensions of a, b, and c, wherein $a = b = \text{about } 112 \text{ \AA}$, $c = \text{about } 110 \text{ \AA}$, $\alpha = \beta = 90^\circ$, and $\gamma = 120^\circ$.

87. (New) A crystal of beta secretase having amino acid sequence SEQ ID NO:1 with the proviso that at least one methionine is replaced with selenomethionine, wherein the crystal has the trigonal space group symmetry $P3_221$ and comprises a unit cell having dimensions of a, b, and c, wherein $a = b = 112 \pm 20 \text{ \AA}$, $c = 110 \pm 20 \text{ \AA}$, $\alpha = \beta = 90^\circ$, and $\gamma = 120^\circ$.

88. (New) The crystal of claim 87 wherein the crystal further comprises an inhibitor.

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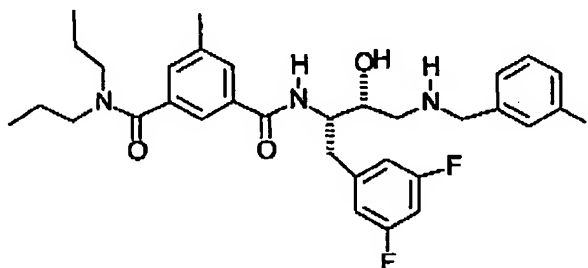
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89. (New) The crystal of claim 88 wherein the inhibitor is



90. (New) A crystal of beta secretase having amino acid sequence SEQ ID NO:1 with the proviso that at least one methionine is replaced with selenomethionine, wherein the crystal has the trigonal space group symmetry $P3_221$ and comprises a unit cell having dimensions of a, b, and c, wherein $a = b = \text{about } 112 \text{ \AA}$, $c = \text{about } 110 \text{ \AA}$, $\alpha = \beta = 90^\circ$, and $\gamma = 120^\circ$.

91. (New) A crystal of beta secretase comprising atoms arranged in a spatial relationship represented by the structure coordinates listed in Table 1.

92. (New) A crystal of beta secretase comprising atoms arranged in a spatial relationship represented by the structure coordinates listed in Table 3.

93. (New) A method for preparing a beta secretase crystal comprising:
preparing purified human beta secretase having amino acid sequence SEQ ID NO:1 in the presence of an inhibitor; and
crystallizing human beta secretase from a solution having a pH of about 3.5 to about 5.5 to provide a crystal according to claim 83.

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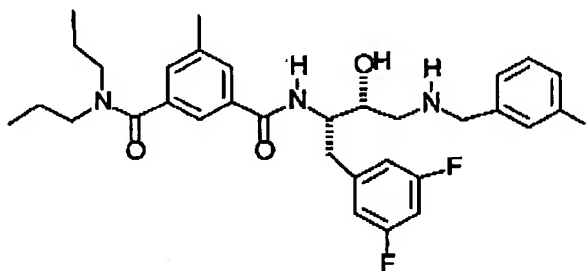
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94. (New) The method of claim 93 wherein the inhibitor is

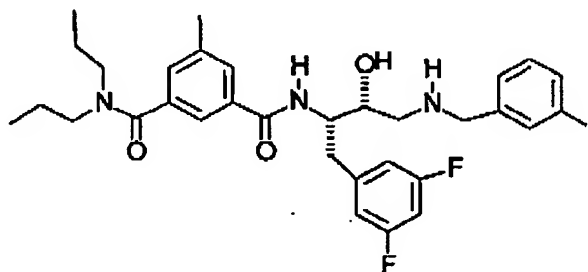


95. (New) A method for preparing a beta secretase crystal comprising:

preparing purified human beta secretase having amino acid sequence SEQ ID NO:1 in the presence of an inhibitor; and

crystallizing human beta secretase from a solution having a pH of about 3.5 to about 5.5 to provide a crystal according to claim 86.

96. (New) The method of claim 95 wherein the inhibitor is



97. (New) A method for preparing a beta secretase crystal comprising:

preparing, in the presence of an inhibitor, purified human beta secretase having amino acid sequence SEQ ID NO:1 with the proviso that at least one methionine is replaced with selenomethionine; and

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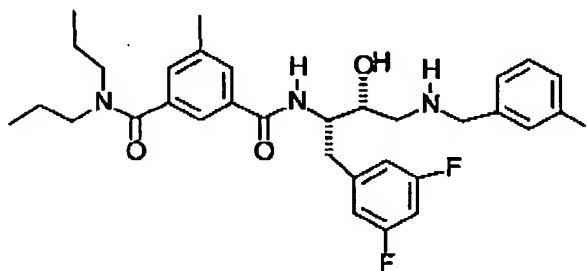
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crystallizing human beta secretase from a solution having a pH of about 3.5 to about 5.5 to provide a crystal according to claim 87.

98. (New) The method of claim 97 wherein the inhibitor is

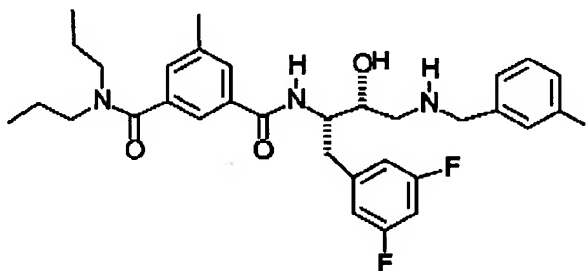


99. (New) A method for preparing a beta secretase crystal comprising:

preparing, in the presence of an inhibitor, purified human beta secretase having amino acid sequence SEQ ID NO:1 with the proviso that at least one methionine is replaced with selenomethionine; and

crystallizing human beta secretase from a solution having a pH of about 3.5 to about 5.5 to provide a crystal according to claim 90.

100. (New) The method of claim 99 wherein the inhibitor is



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101. (New) A method for crystallizing human beta secretase comprising:
preparing purified human beta secretase at a concentration of about 1 mg/ml to about 80 mg/ml in the presence of a substrate; and
crystallizing human beta secretase from a solution comprising about 5% by weight to about 50% by weight of a glycol and having a pH of about 3.5 to about 5.5.
102. (New) The method of claim 101 wherein the solution further comprises a salt present in about 0.001 M to about 0.5 M concentration.
103. (New) The method of claim 101 wherein the solution further comprises a buffer present in about 10 mM to about 200 mM concentration.
104. (New) The method of claim 101 wherein the solution further comprises up to about 20% by weight DMSO.
105. (New) A method for crystallizing human beta secretase comprising:
preparing purified human beta secretase at a concentration of about 18 mg/ml to about 40 mg/ml in the presence of a substrate; and
crystallizing human beta secretase from a solution comprising about 17% by weight to about 22% by weight of a glycol, a buffer present in about 10 mM to about 200 mM concentration, and having a pH of about 4.0 to about 4.7.
106. (New) The method of claim 105 wherein the solution further comprises a salt present in about 0.001 M to about 0.5 M concentration.
107. (New) The method of claim 105 wherein the solution further comprises up to about 20% by weight DMSO.